Moreover, the expression levels of these genes (e.g. CD44, PTRZ, GLI3, NTRK2) were significantly modulated upon GW4869 treatments. In conclusion, our study highlights the importance of the exosomes in the inter-clonal communication and suggests that interfering with the exosome biogenesis may be a valuable strategy to inhibit cell motility in PDHGG.

HGG-44. UNRAVELING AND TARGETING THE STEM-REGULATORY NETWORK DRIVING INVASION IN DIFFUSE HEMISPHERIC GLIOMA, H3G34-MUTANT

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Diffuse hemispheric glioma H3G34-mutant (DHG-G34) is a pediatric-type high-grade glioma affecting children and young adults. Despite surgery and radio/chemotherapy, patients have a dismal prognosis. The intratumoural heterogeneity and the high infiltrative nature of DHG-G34 cells limit the development of effective therapies. Analysing single-cell RNA sequencing data from a publicly available dataset, we identified a large and distinct sub-population of cells displaying high stem and low differentiation marker expression levels. Gene ontology analyses revealed a gene signature related to cell migration/invasion. This observation is supported by our data on in vitro 3D invasion assay and in vivo orthotopic xenograft models, showing that DHG-G34 disseminating cells are characterised by high expression level of the stem-cell marker NESTIN and low expression level of the differentiation marker GFAP. Following these findings, we developed high-throughput cell-based assays with the aim to screen a library of 1300 FDA-approved compounds and identify drugs able to induce DHG-G34 cell differentiation and inhibit their invasive phenotype. The screen, a co-immunofluorescence assay for NESTIN and GFAP, followed by dose response assays on 3D growth and 3D invasion, led to the identification of 3 FDA-approved drugs, the MEK inhibitor Cobimetinib and 2 HMG-CoA reductase inhibitors, Rosuvastatin and Pitavastatin. These 3 drugs potently induced cell differentiation (decreased Nestin and increased GFAP expression) and inhibited invasion with minimal effect on the proliferation of our DHG-G34 cell line. We are currently extending these findings to additional patient-derived DHG cell lines and we are using these drugs and different omics and imaging technologies to characterize the regulatory networks associated to DHG-G34 stemness, (de)-differentiation and invasiveness. Our work may lead to the identification of new therapeutic approaches for targeting the stem/invasive properties of these aggressive diseases.

HGG-45. CHARACTERIZATION OF SPINAL DIFFUSE MIDLINE GLIOMAS, H3 K28M-MUTANT

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Diffuse midline gliomas (DMGs) are malignant gliomas that arise in the midline structures of the central nervous system. Due to their aggressive and diffuse growth and a two-year survival rate of less than 10%, DMGs are assigned to CNS WHO grade 4. Depending on the localization, median age of patients is about 11–20 years. Genetically, most tumors are defined by a K28M-mutation in one of the highly homologous genes encoding histone protein H3. Since DMGs most frequently occur in pons and thalamus, comparatively little is known about spinal DMGs. Therefore, we histologically, molecularly, and clinically characterized spinal DMGs and analyzed, in which aspects they differ from DMGs of other localizations. Our cohort currently consists of 25 spinal DMGs and 40 pontine/thalamic reference cases. Histological, immunohistochemical and molecular analyses (DNA methylation, DNA panel sequencing) were done from FFPE tissue. Spinal DMGs were histologically very heterogeneous, both regarding different areas of single tumors as well as in comparison to other spinal and reference cases. First cluster analyses of DNA methylation data indicated a separation into three main clusters enriched for pontine, thalamic or spinal cases. The cluster enriched for spinal cases contained many tumors from elderly patients. Overall, mean age of patients with spinal DMGs was 28 years. Patients were significantly older than those with pontine DMGs. 19/20 spinal DMGs were H3-3A K28M-mutant, while one tumor had an H3-2B mutation. 4/19 (21%) spinal DMGs had mutations in FGFR1, and 6/10 (60%) in NF1. Three tumors had KRAS or BRAF mutations. In summary, first analyses suggest slight histological differences of spinal DMGs compared to DMGs of other localizations. Preliminary cluster analyses of DNA methylation data showed an enrichment of clusters for different localizations. About one third of spinal DMGs had mutations in a gene associated with the MAPK-signaling pathway.

HGG-46. INTER AND INTRA-TUMOR HETEROGENEITY OF PEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMA REVEALED BY HIGH-DIMENSIONAL SINGLE-CELL PROTEOMICS

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Paediatric-type diffuse high-grade gliomas (PDHGG) are aggressive brain tumors, affecting children and young adults, with no effective treatments. A main constraint to the development of effective treatment is associated with their highly heterogeneous nature. In order to further dissect their intra and inter tumor heterogeneity, we exploited the mass cytometry technology, an advanced -OMIC approach that, by using metal-tagged antibodies, allows the simultaneous measurement of more than 40 markers, at single-cell level. Here we characterized 8 primary cell lines derived from diffuse pediatric-type high-grade glioma H3-wildtype (DHGG-WT), Diffuse hemispheric glioma H3G34-mutant (DHG-G34) and Diffuse midline glioma H3K27-altered (DMG-K27) patients. The adopted antibody panel was set to recognize antigens expressed by brain and tumor cells, including H3K27M and H3.3G34R variants, and it highlighted important intra- and inter- tumor heterogeneity in the expression of the 16 considered markers. Of these, CD56, CD44, CD29 and NESTIN were more expressed in the hemispheric cell lines, while CD90 was more expressed in the pontine. Even if there was not always a concordance between CyTOF and mRNA expression data from cell lines and tumor samples (e.g. CD90 and GFAP), CyTOF data were in line with the immunohistochemistry analysis for GFAP, whose expression was significantly higher in H3.1K27 compared to H3.3K27. The UMAP analysis allowed us to identify 10 cell clusters, with very minimal overlap between hemispheric and pontine location subgroups and with a peculiar antigenic profile, whose abundance strongly varied according to the mutational subgroups. For example, while the G34 subgroup was enriched for cluster 9 (CD29/CD63/CD56/PDGRFa), the H3.1K27 was enriched for cluster 3 (H3K27M/CD90/CD63/CD56) and cluster 4 (H3K27M/CD63/CD90/CD56/GFAP). In conclusion, single-cell mass cytometry reveals a significant inter and intra-tumoral heterogeneity at protein level, dependent on the molecular alterations. This approach could contribute to the identification of new clinically relevant biomarkers for PDHGG.

HGG-47. COMPARATIVE ANALYSIS OF THE HISTONE H3 MUTANT PROTEIN INTERACTOME LANDSCAPE IN PAEDIATRIC HIGH-GRADE GLIOMAS

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There have been no significant improvements in the treatments for childhood and adolescent High-Grade Glioma (pHGG) and Diffuse Intrinsic Pontine Glioblastoma (DIPG), which have a very poor prognosis. These cancers harbour mutations affecting histone 3 (H3) proteins, 80% of DIPGs harbour histone H3.1 and H3.3 K27M somatic mutations whilst 30% of pHGGs exhibit H3.3 G34R or G34V mutations. Several studies have highlighted the epigenetic changes associated with these mutations, however their precise role in tumourigenesis is still unknown. We hypothesize that H3 mutations promote an aberrant interaction landscape and analysis of these interactome will highlight important pathophysiological consequences in these tumours. Two different affinity chromatographic proteomic analyses